SUPPLEMENT ARTICLE

Survey of Clinical Laboratory Practices for Parasitic Diseases

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To gain knowledge about laboratory testing practices for parasitic diseases, in 2000 we surveyed 562 laboratories in 9 US states, and 455 (81%) responded. Most laboratories (59%) indicated that they send specimens off site for parasite screening, and most laboratories (89%) did not routinely test fecal specimens for *Cryptosporidium* species, *Cyclospora cayetanensis,* or microsporidia, unless testing for these organisms was specifically requested by a physician. Only 39 laboratories offered serological testing for *Toxoplasma gondii,* and most (78%) that had their results confirmed did so at national commercial laboratories rather than a *Toxoplasma* reference laboratory. Because most clinical laboratories do not routinely test fecal specimens for *Cryptosporidium* species, *C. cayetanensis,* or microsporidia, physicians must request specific testing for these organisms when they are clinically suspected; because of this lack of routine testing, it is difficult to estimate the true burden of disease due to these organisms.

A report from the Centers for Disease Control and Prevention (CDC) indicated that parasitic diseases cause 3% of foodborne illnesses and 21% of foodborne illness–related deaths in the United States [1]. In national surveillance data reported during 1993–1997, parasitic diseases caused 2% of foodborne outbreaks and 5% of illnesses associated with foodborne outbreaks [2]. Diseases caused by parasitic organisms are also responsible for the largest single category of reported drinking water (35%) and recreational water (50%) outbreaks in the United States [3]. One foodborne parasite, *Toxoplasma gondii,* is the leading cause of CNS infection among persons with AIDS [4]. In addition, *T. gondii* causes up to 4000 congenital infections per year in the United States—these infections can lead to blindness, learning disabilities, and mental retardation in children. Another parasite that can be both foodborne or waterborne, *Cryptosporidium parvum,* can also cause severe illness in immunocompromised persons [5]. Parasitic diseases such as giardiasis and cryptosporidiosis acquired from food and water are also a major cause of chronic diarrhea acquired by US travelers to foreign countries [6]. However, parasitic diseases often go undiagnosed, because laboratory tests for specific parasitic organisms are not requested by health care providers and because stool specimens are often not obtained and sent for examination.

To gain knowledge about laboratory testing practices for parasitic diseases in the United States, staff working with the Emerging Infections Program of the CDC conducted a survey in 2000 of all clinical laboratories in the surveillance area of the Foodborne Dis-

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eases Active Surveillance Network (FoodNet). We present the results of the parasitic diseases component of the laboratory survey.

METHODS

FoodNet, which is a collaborative project of the CDC, the US Department of Agriculture Food Safety and Inspection Service, the US Food and Drug Administration, and selected state health departments, conducts active surveillance for diseases that can be foodborne. Epidemiologic studies are also conducted to help public health officials better understand the source and spread of foodborne diseases in the United States. In 1996, FoodNet surveillance began in 5 states: counties in California (Alameda, Contra Costa, Marin, San Francisco, San Mateo, Santa Clara, Solono, and Sonoma) and Connecticut (Fairfield, Hartford, Litchfield, Middlesex, New Haven, New London, Tolland, and Windham) and the entire states of Georgia, Minnesota, and Oregon. Counties in New York (Albany, Columbia, Genesee, Greene, Livingston, Monroe, Montgomery, Ontario, Orleans, Rensselaer, Saratoga, Schenectady, Schoharie, Wayne, and Yates) and Maryland (Anne Arundel, Baltimore, Baltimore City, Carroll, Harford, Howard, Montgomery, and Prince George's) were added in 1998, counties in Tennessee (Cheatham, Davidson, Dickson, Hamilton, Knox, Robertson, Rutherford, Shelby, Sumner, Williamson, and Wilson) were added in 1999, and counties in Colorado (Adams, Arapahoe, Denver, Douglas, and Jefferson) were added in 2000. The population of the FoodNet surveillance area (also known as "FoodNet sites") is 34.0 million persons, or 12% of the US population, according to 1999 census estimates.

FoodNet laboratory surveys conducted in 1996, 1997, and 2000 collected information about pathogen testing, the volume of testing, and testing practices from all clinical laboratories in FoodNet sites that process stool samples for parasitic, bacterial, and/or viral pathogens. Only the results of the year 2000 laboratory survey for parasitic diseases are presented in this article.

Questionnaires were mailed to each laboratory during February–March 2000. The questionnaire was 11 pages long and took ~45 min to complete. Responses regarding the volume of testing are given for the calendar year 1999. The parasitic diseases portion of the questionnaire was 2.5 pages long and included questions regarding when and whether laboratories tested for parasites, the number of specimens submitted, positivity rates, types of tests, and staining procedures. In addition, for laboratories that perform parasitic testing, questions addressed testing practices for ova and parasites and specific tests for *Cryptosporidium* species, *Cyclospora cayetanensis,* and microsporidia and *T. gondii* serological testing. Data from all sites are included, except those from Maryland, which were not available at the time of the analysis. Analyses were performed with EpiInfo [7] and SAS [8] software. We conducted the study in accordance with guidelines for human research as specified by the US Department of Health and Human Services.

RESULTS

Of 562 laboratories surveyed, 455 (81%) responded to at least some of the questions. The responding laboratories indicated that 492,650 fecal specimens were submitted for parasitic testing during 1999 (median, 293 specimens/laboratory; 25th percentile, 100; 75th percentile, 858; range, 0–69,700). Of 405 responding laboratories, 54% indicated that fecal specimens for ova and parasite testing were received routinely (i.e., >80% of the time) in preservative, as feces in a container not in transport media (34% of 400 responding), as feces in transport media (22% of 394 responding), and, less commonly, as a rectal swab specimen (3% of 391 responding). Most of the laboratories (59% of 416 responding) indicated that they send specimens off-site for parasitic screening.

Procedures for ova and parasite testing varied. Many laboratories used a stool concentration (58% of 403 responding); some used a trichrome staining method (48% of 403 responding), an acid-fast staining method (41% of 403 responding), wet mounts before concentration or sedimentation (36% of 407 responding), parasitologic examination of tissue and fluid samples (35% of 399 responding), and/or immunoassay (e.g., direct immunofluorescence [IFA] or EIA) for antigen detection (35% of 400 responding). Few laboratories conducted molecular diagnostic tests (1% of 400 responding—only 3 laboratories) or parasitic culture or inoculation into experimental animals (1% of 390 responding—only 5 laboratories).

Laboratories did not routinely test fecal specimens for *Cryptosporidium* species, *C. cayetanensis,* or microsporidia (<11% did so for ova and parasite requests and <1% for all liquid fecal specimens) unless testing for a specific organism was requested by a health care provider (table 1). The most common testing methods that the surveyed laboratories used for these organisms are shown in table 2. Of the 492,650 total fecal specimens, 135,960 (28%) were examined for *Cryptosporidium* species, and 707 (0.5%) yielded positive results; of 35,544 specimens (7% of total fecal specimens) examined for *C. cayetanensis,* 29 (0.1%) yielded positive results; and, of 3471 specimens (1% of total fecal specimens) examined for microsporidia, 34 (1%) yielded positive results.

Of 398 laboratories indicating that they performed serological testing for *T. gondii,* 39 (10%) offered laboratory testing on site. All 39 laboratories offered *T. gondii*–specific immunoglobulin or IgG testing. The methodologies used were EIA (85%) and IFA (15%). Of the 39 laboratories, 23 performed

Table 1. Circumstances in which laboratories test for *Cryptosporidium* species, *Cyclospora cayetanensis,* and microsporidia, FoodNet laboratory survey, 2000.

Specimen, testing protocol	Percentage of laboratories that test for category of organism		
	Cryptosporidium species (n = 410)	*C. cayetanensis* (n = 411)	Microsporidia (n = 409)
All liquid fecal specimens	0.2	0.5	0.0
All liquid fecal specimens for O&P	0.7	0.0	0.0
All fecal specimens for O&P	10.7	6.3	0.7
All fecal specimens from HIV-positive persons for O&P	0.2	0.0	0.2
Fecal specimens when testing for the specific organism is requested	38.5	27.3	13.9
Other/unknown	2.2	1.9	1.2
Fecal specimens tested for the organism on site under some circumstances			
Yes	52.5	36.0	16.0
No	47.5	64.0	84.0

NOTE. O&P, ova and parasites.

T. gondii–specific IgM tests (78% by EIA and 22% by IFA). Only 1 laboratory performed PCR for *T. gondii* DNA. All 39 laboratories accepted serum or plasma samples for testing. A total of 27 laboratories answered the question regarding the acceptance of CSF; of these, 7 (26%) accepted such samples for testing. A total of 25 laboratories answered the question regarding the acceptance of amniotic fluid or tissue; of these, 1 (4%) accepted such samples for testing. Of all 39 laboratories testing for *T. gondii,* 29 answered the questions regarding the number of specimens tested, indicating a total of 15,198 specimens underwent *T. gondii* immunoglobulin or IgG testing in 1999, of which 537 (3.5%) yielded positive results. The 23 laboratories that test for *T. gondii* IgM received 5129 specimens, of which 43 (0.8%) tested positive. Of 27 laboratories providing information about where they send *T. gondii* specimens for confirmatory testing, 21 (78%) sent specimens to national commercial laboratories. A total of 13 laboratories were listed for confirmatory testing, 9 (69%) of which were national commercial laboratories.

DISCUSSION

It is apparent from the year 2000 laboratory survey that most laboratories do not routinely test stool specimens for parasites. As a result, parasitic diseases are likely to be underdiagnosed and underreported in the United States. Our results also indicate that health care providers must specifically request tests for *Cryptosporidium* species, *C. cayetanensis,* and microsporidia because they are not included in a routine ova and parasite examination.

Likewise, the results of a 1996 survey in Connecticut of the factors that influence testing for *Cryptosporidium* species indicated that most laboratories examined stools specifically for *Cryptosporidium* species only on physician request [9]. Similar findings were reported in the 1997 FoodNet laboratory survey [10]. The Connecticut survey further concluded that higher rates of *Cryptosporidium* positivity occurred with the use of monoclonal antibody methods, the use of ⩾2 staining procedures, and the testing of stool specimens even if it was not requested by a physician. In the FoodNet 2000 laboratory survey, as in the Connecticut survey and the 1997 FoodNet laboratory survey, acid-fast staining was still the most predominant method used, which also might be a factor that contributes to the low positivity rate of testing for *Cryptosporidium* species

Parasitic diarrheal diseases often have an incubation period of ~1 week, can last ⩾1 week, and may be recurrent. In such circumstances, a high index of suspicion is required to request tests for specific parasitic diseases. Although it was not researched in the FoodNet 2000 survey, giardiasis is another illness that should be considered when prolonged or intermittent diarrhea occurs.

The FoodNet laboratory survey is subject to limitations. The FoodNet surveillance area is not a statistical sample of the US population; therefore, laboratory practices may differ in other areas. Although the response rate was good (81%), the volume of testing and other related information was not available from laboratories that did not participate in the survey for comparison with laboratories that did participate.

Only 10% of the laboratories in FoodNet sites perform antibody detection tests for *T. gondii,* and most of these laboratories use national commercial laboratories for the confirmation of results instead of a *T. gondii* reference laboratory. The *T. gondii* IgM test is often used to help determine whether an acute infection has occurred in a pregnant woman. An acute

Table 2. Tests used to examine stool samples for *Cryptosporidium* species, *Cyclospora cayetanensis*, and microsporidia, FoodNet laboratory survey, 2000.

Type of test[a]	Percentage of laboratories testing for organism		
	Cryptosporidium species (n = 213)	*C. cayetanensis* (n = 142)	Microsporidia (n = 60)[b]
Wet mount			
Not stained	7.5	9.9	NA
Stained with iodine or other temporary stains	8.0	15.5	NA
Acid-fast staining	52.5	69.0	1.7
Direct immunofluorescence	23.0	NA	NA
EIA	17.3	NA	NA
Safranin staining	NA	2.1	NA
UV fluorescence	NA	7.0	NA
Chromotrope and modified trichrome staining	NA	NA	53.3
Calcofluor white staining	NA	NA	15.0
Polymerase chain reaction	0.0	0.0	1.7
Auramine-rhodamine staining	0.9	0.0	0.0
Other	0.0	1.4	8.3

NOTE. NA, not applicable.

[a] Laboratories may have used >1 testing method.

[b] Only 48 of 60 laboratories that tested for microsporidia listed the testing methods used.

infection in a pregnant woman puts the fetus at risk for congenital toxoplasmosis and may require treatment with potentially toxic medications (e.g., pyrimethamine and sulfadiazine). Some *T. gondii* IgM tests have shown decreased specificity for acute infection because of both false-positive results and a persistence of the IgM response [11]. Therefore, positive *T. gondii* IgM test results should be confirmed by a qualified *T. gondii* reference laboratory, where additional tests (e.g., the differential agglutination test [AC/HS] [12] or avidity test [12–14]) are available to help determine when (i.e., before or during pregnancy) a woman became infected with *T. gondii*. We are aware of only 2 laboratories in the United States that offer ⩾1 of these additional tests (Toxoplasma Serology Laboratory, Research Institute, Palo Alto Medical Foundation, Palo Alto, CA, which offers reference laboratory testing and clinical consultation and interpretation; and Focus Technologies, Cypress, CA). Additional information about testing, pregnancy, and congenital toxoplasmosis can be found in a recent review [15].

Our results reveal that the burden for many parasitic diseases is often difficult to estimate, because US laboratories do not routinely test for them and physicians often do not request the specific tests required for laboratory diagnosis. Nevertheless, the FoodNet laboratory survey has provided valuable insight into the percentage of laboratories that test for several key parasitic organisms and the types of tests that are used.

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